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Effects of source/sink manipulation on grain zinc accumulation by winter wheat genotypes

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ABSTRACT

Agronomy practices aimed at wheat (*Triticum aestivum* L.) grain Zn biofortification are important to alleviate Zn deficiency in humans, especially for those resource-poor people owing to subsistence on diets dominated by cereal-based foods with low concentrations and bioavailability of Zn. To promote understanding of source-sink flow processes affecting biofortification of wheat with Zn, effects of different source/sink manipulations on grain Zn accumulation by wheat were examined in pot and field at two locations. Treatments included foliar applications of deionized water, sucrose, $ZnSO_4$, sucrose + Zn, defoliation, spike shading or partial removal of spikes. Results showed sucrose + Zn significantly increased grain Zn concentration more than Zn alone. Grain Zn concentration with sucrose + Zn averaged 66 mg kg^{-1} for 'Kenong 9204' and 59 mg kg^{-1} for 'Liangxing 99' in pot, and ranged from 42 to 58 mg kg^{-1} for 'Liangxing 99', 'Jinan 17', 'Jimai 20', 'Jimai 22', and 'Luyuan 502' under field, approaching the field-grown target 60 mg kg^{-1} proposed by World Health Organization. Molar ratios of phytic acid/Zn and phytic acid × Ca/Zn of 'Jinan 17', 'Jimai 20' or 'Luyuan 502' with sucrose + Zn were reduced to be < 15 and < 200, respectively, suggesting higher Zn bioavailability. Defoliation or spike shading decreased grain weight, Zn concentration and content. It is concluded that grain Zn accumulation of wheat can be affected by the source-sink relationship of Zn and/or carbohydrate, and the foliar spray 'sucrose + Zn' is recommended for increasing concentration and bioavailability of Zn in wheat grains.

Key words: Biofortification, carbohydrate, cereals, micronutrient, remobilization, *Triticum aestivum*.

INTRODUCTION

Wheat (*Triticum aestivum* L.) grains contain inherently too low Zn to meet daily human requirement, particularly when wheat crops are grown on Zn-deficient soils (Cakmak, 2008). In addition, wheat is rich in anti-nutritional compounds such as phytic acid and phenolic compounds that reduce biological availability of Zn in the human digestive tract (Cakmak et al., 2010b). It is therefore of great interest to increase Zn concentrations as well as bioavailability in wheat grains for human health benefits.

The movement of nutrients into grains is affected by the source-sink relationship at the grain-filling stage. Kutman et al. (2012) found that Zn remobilization rather than root uptake is critical for Zn accumulation in wheat grains when Zn availability in soil is restricted at the grain-filling stage. Foliar Zn application is much more effective than soil Zn application in Zn enrichment of wheat grains (Wang et al., 2012; Zhang et al., 2012b). The concentrations of Zn in wheat grains were positively correlated with foliar Zn rates (Zhang et al., 2012b). All these results suggested that Zn translocation to grains or grain sink strength is not a limiting factor and the grain Zn concentration is most probably limited by source supply (Zhang et al., 2012b). However, increasing Zn concentration in the culture solution from 10 to 100 μmol L^{-1} failed to result in a 10-fold increase in the wheat grain Zn concentration, perhaps as a result of saturating the membrane transporters during phloem loading (Pearson et al., 1996b). Wang et al. (2011) further reported that two barriers of Zn transport into wheat grains existed: 1) between the stem tissue rachis and the grain, and 2) between the maternal and the filial tissues in the grain. Therefore, the extent of source/sink limitation of grain Zn concentration and regulatory mechanisms need further studies.

The carbohydrate status in plants can influence the Zn transport into the grain. Pearson et al. (1996b) observed that the depletion of sucrose in the cultured ears of wheat through maintaining them in the dark decreased the transport of Zn to the grain, perhaps as a result of a decrease in mass flow of carbohydrates within the phloem. Due to the limitation of the grain sink capacity, sucrose at high supply rates may be accumulated in the peduncle and chaff, resulting in stomatal closure, the abatement of transportation by the xylem, and finally a decreased accumulation of micronutrients (including Zn) in grains (Ma et al., 1996). Recent studies showed the grain Zn concentration significantly decreased with increasing sucrose supply to detached ears, due to a dilution effect resulting from the increase in grain weight (Zhang et al., 2012a; Liu et al., 2014). However, it is unknown whether exogenous sucrose supply (with/without Zn) affects grain Zn accumulation of wheat grown in soil.

Dry weight accumulation of grains is determined by the source-sink relationship of crop photoassimilates (Zhang et al., 2012a). Ma et al. (1996) and Wang et al. (1997) investigated the source-sink limitation for wheat grain growth by partial spikelet removal and defoliation. In their experiments, partial spikelet removal reduced the grain number and total sink size, and increased the source-to-sink ratio. Defoliation after anthesis decreased carbohydrate supply, and subsequently decreased single-grain weight and grain number. However, these studies analyzed only the grain growth and DM accumulation but not the accumulation dynamics of nutrients.

This study aimed to examine the effects of source and sink manipulations on grain Zn accumulation of wheat (*Triticum aestivum* L.) cultivars under controlled-environment and field conditions. Here we reduced carbohydrate source through defoliation and spike shading or increased it by reducing spike numbers after anthesis. Effects of foliar applications of sucrose and Zn alone or in combination on Zn accumulation and bioavailability of wheat grains were also studied. Zinc bioavailability in grains was estimated using molar ratios of phytic acid to Zn and phytic acid×Ca/Zn (Morris and Ellis, 1982; Ellis et al., 1987; Ryan et al., 2008). The results from these experiments provide novel information for guiding agronomic practices to enhance Zn biofortification of wheat grains.

MATERIALS AND METHODS

Glasshouse experiment ('Kenong 9204' and 'Liangxing 99')

Two winter wheat cultivars (*Triticum aestivum* L., 'Kenong 9204' and 'Liangxing 99' classified as high-yielding cultivars and widely cultivated in northern China) were grown in ceramic pots containing 4.0 kg air-dried calcareous soil in a glasshouse from December 2009 to May 2010. The basic properties of the soil were as follows: pH 8.0 (1:2.5 w/v in water), organic matter 13 g kg^{-1}, total N 0.5 g kg^{-1}, Olsen-P 5.2 mg kg^{-1}, exchangeable K 90 mg kg^{-1}, diethylenetriamine pentaacetic acid (DTPA)-extractable Zn 2.3 mg kg^{-1}. Before sowing, the following nutrients were homogeneously incorporated into the soil (per kg dry soil): 150 mg N as urea (70% applied as basal dose and 30% as the top-dressing at the jointing stage), 65.5 mg P in the form of calcium superphosphate containing 12% P_2O_5, 124.5 mg K in the form of potassium sulfate for agriculture containing 50% K_2O and 5 mg Zn in the form of $ZnSO_4{\cdot}7H_2O$ with purity ≥ 99.5%. Fifteen seeds per wheat cultivar were sown in each pot and seedlings were thinned to 10 after 9 d.

The experiment was arranged in a complete randomized design. There were seven treatments in four replicates for 'Kenong 9204', and four foliar application treatments in three replicates for 'Liangxing 99' (Table 1). All solutions containing 0.01% (v/v) Tween 20 as a surfactant were foliar-applied at 80 mL per pot after sunset. When spraying, the soil in pots was covered with plastic films to prevent possible contamination by solutions. The foliar applications were conducted four times at 1-wk intervals from 5 d after flowering. At maturity, all plants were harvested and grains were separated manually from straw.

Table 1. Source/sink treatments on winter wheat grown under glasshouse or field conditions.

Treatments		Nutrient concentration of foliar spray or other treated methods after wheat anthesis
T1	Control	Deionized water
T2	Sucrose	3.0% (w/v)
T3	$ZnSO_4·7H_2O$ (Zn)	0.3% (w/v) (0.4% for the last experiment)
T4	Sucrose + Zn	3.0% and 0.3% (w/v) (0.4% Zn for the last experiment)
T5	Partial spike removal	Removing stunting spikes from tillers
T6	Spike shading	Covering all spikes with water-proof bags
T7	Defoliation	Removing all leaf blades from wheat after anthesis

Field experiment ('Liangxing 99') at Quzhou experimental station during the 2009-2010 cropping season

A field experiment was conducted at Quzhou experimental station (36.9° N, 115.0° E) in Hebei province of China by only one cropping season of winter wheat (2009-2010). The soil characteristics were calcareous alluvial soil typical of the North Plain of China, pH 8.3 (1:2.5 w/v in water) and 14 g kg^{-1} organic matter. The DTPA-extractable Zn was 3.2 mg kg^{-1} before sowing. The climatic conditions of the experiment have been previously reported by Zhang et al. (2013).

Wheat 'Liangxing 99' was planted in a complete randomized design with four replicates. The amount of N application is the commonly applied 300 kg N ha^{-1} as urea. Other detailed information about fertilizer application and crop husbandry were previously described by Xue et al. (2014). The treatments (T1-T7) were same as those applied to 'Kenong 9204' in the glasshouse (Table 1). All solutions containing 0.01% (v/v) Tween 20 as a surfactant were applied at 600 L ha^{-1} (60 mL m^{-2}) after sunset. The area of each replicate for treatments T1-T4 was 10 m^2 and 1 m^2 each for T5-T7. At maturity, plants from T1-T4 were harvested manually in a 2 m^2 area. For T5-T7, all plants were harvested manually.

Field experiment ('Jinan 17', 'Jimai 20', 'Jimai 22' and 'Luyuan 502') at Yinmaquan experimental station during the 2014-2015 cropping season

Another field experiment was conducted during the 2014-2015 cropping season at Yinmaquan Experimental Station (36°43' N, 117°5' E; 48 m a.s.l.), Shandong Academy of Agricultural Sciences, China. The area is a typical continental and warm climate, with an annual mean temperature of 13.6 °C and a long-term mean annual rainfall of 625 mm. The soil at the site was classified as sandy loam, with a pH of 7.8. The top 20 cm of the soil contained 19 g kg^{-1} organic matter, 49 mg kg^{-1} water-hydrolysable N, 24 mg kg^{-1} Olsen-P, 162 mg kg^{-1} exchangeable K and 1.5 mg kg^{-1} DTPA-extractable Zn. All the P fertilizer (52 kg P ha^{-1}, supplied as calcium superphosphate), K fertilizer (83 kg K ha^{-1}, supplied as potassium sulfate), Zn fertilizer (6.8 kg Zn ha^{-1}, supplied as $ZnSO_4·7H_2O$) and 113 kg N ha^{-1} (supplied as urea) were evenly broadcast and incorporated into the upper 20 cm of the soil prior to wheat sowing. The other half of the N was top-dressed as urea at the jointing stage.

The experiment was a split-plot design, and consisted of three foliar spray treatments (subplot) and four cultivars (main plot) in four replicates. Four winter wheat cultivars were 'Jinan 17', 'Jimai 20', 'Jimai 22' and 'Luyuan 502'. 'Jinan 17' is a high-quality strong gluten cultivar, suitable for making bread. 'Jimai 20' is suitable for making both bread and noodles. 'Jimai 22' is a high-yielding wheat cultivar and sown on the largest area in contemporary China. 'Luyuan 502' is also a high-yielding wheat cultivar, which is widely cultivated in northern China. The three foliar treatments were T1, T3 and T4 as shown in Table 1. Compared to the first field study, the concentration of $ZnSO_4·7H_2O$ in T3 and T4 was increased from 0.3% to 0.4% (w/v) in this experiment. All foliar solutions contained 0.01% (v/v) Tween 20 as a surfactant and the application rate was increased from 600 to 900 L ha^{-1} (equal to 90 mL m^{-2}) after sunset. The area of the main plot was 80 m^2, and of subplot 10 m^2 each. At maturity, wheat plants from T1, T3 and T4 were harvested manually in a 1 m^2 area in the center of each split-plot.

Nutrient analysis

After being washed with deionized water, grain samples were dried at 60-65 °C for 72 h. The dried samples were ground with a stainless steel grinder (RT-02B, Chinese Taipei) and digested with HNO_3-H_2O_2 in a closed microwave digester (CEM, Matthews, North Carolina, USA). The concentrations of nutrients in the digests were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, OPTIMA 3300 DV, Perkin-Elmer, Waltham, Massachusetts, USA). A reference grain sample IPE556 from Wageningen University was included in each batch to ensure analytical quality. Phytate P concentration in the Yinmaquan field experiment was analyzed according to the method of Haug and Lantzsch (1983). Phytate P was converted to phytic acid (PA) by dividing by 0.282 to calculate molar ratios of PA/Zn and PA × Ca/Zn.

Statistical analysis

Data from the single-factor completely randomized design and split-plot design experiments were subjected to ANOVA using SAS software (SAS 8.0, SAS Institute, Cary, North Carolina, USA) and mean values were compared by the least significant difference (LSD) at the 5% level.

RESULTS

Yield traits, grain Zn concentrations and contents of wheat from pot experiments

Compared with the control (T1), only defoliation (T7) significantly decreased grain yield and biomass while other treatments (T2-T6) did not (Table 2). Thousand kernel weight (TKW) varied from 28.9 to 49.4 g. Spike shading (T6) significantly decreased the TKW compared with T1, while T7 led to a further decrease compared to T6. Harvest index (HI) varied from 47.3% to 54.6%, with T7 treatment also being the lowest. Compared with partial spike removal, spike shading and defoliation decreased grain yield, biomass and TKW gradually. In addition, defoliation decreased kernels per spike and HI (Table 2).

For 'Kenong 9204', foliar spray of Zn increased grain Zn concentration by 135% and 211% for T3 and T4, respectively, compared with the T1 treatment while other treatments did not (Table 3). Grain Zn concentration increased from 21.1 to 49.6 mg kg^{-1} by Zn-only treatment, and further increased to 65.7 mg kg^{-1} by the sucrose + Zn spray. Grain Zn contents were 123% and 217% higher in T3 and T4, respectively, but 44% and 57% lower in T6 and T7, respectively, and not affected by T2 and T5 significantly, as compared with T1. For 'Liangxing 99', both grain Zn concentration and content were in the order of T1 < T2 < T3 < T4 (Table 3). The foliar spray of sucrose + Zn (T4) led to the highest Zn concentration (59.0 mg kg^{-1}), and increased grain Zn content by 345%, compared with T1.

Yield traits, grain Zn concentrations and contents of wheat 'Liangxing 99' under field conditions at Quzhou

Compared with the control (T1), foliar treatments (T2-T4) had nonsignificant effects on grain yield and TKW (Table 4). While T5 significantly increased TKW, T6 and T7 significantly decreased TKW. The grain Zn concentration was increased from 35.0 mg kg^{-1} in the control to 38.7 mg kg^{-1} by the foliar spray of Zn alone, and increased further from 38.7 to 42.2 mg kg^{-1} by spray of sucrose + Zn combined solution. Compared with T1, other treatments (T2, T5, T6

Table 2. Effects of different source/sink treatments on yield parameters of two wheat cultivars in the pot experiment.

Cultivars	Treatments		Grain yield	Biomass	Kernels	TKW	HI
			g pot^{-1}	g pot^{-1}	nr spike^{-1}	g	%
Kenong 9204	T1	Deionized water	15.9ab	29.6ab	35.0abc	45.5ab	53.7a
	T2	Sucrose	15.8ab	29.9ab	31.9bc	49.4a	52.3a
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	15.5ab	29.1ab	36.7ab	42.4bc	53.4a
	T4	Sucrose + Zn	16.6a	31.5a	35.3abc	47.2ab	52.8a
	T5	Partial spike removal	18.3a	33.6a	37.3a	49.2ab	54.6a
	T6	Spike shading	13.8b	26.5b	38.5a	36.1c	52.0a
	T7	Defoliation	8.8c	18.7c	31.2c	28.9d	47.3b
Liangxing 99	T1	Deionized water	15.0ab	28.8ab	37.0a	41.1a	52.0ab
	T2	Sucrose	14.3b	27.4b	37.0a	38.8a	52.2ab
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	16.9a	31.4a	41.0a	41.0a	53.9a
	T4	Sucrose + Zn	16.1ab	31.3ab	42.0a	38.0a	51.3b

TKW: Thousand kernels weight; HI: harvest index.
Values are means of four and three replicates for 'Kenong 9204' and 'Liangxing 99', respectively.
Values in the same column per cultivar followed by different lower-case letters are significantly different among the treatments according to Fisher's Protected LSD test ($P \leq 0.05$).

Table 3. Effects of different source/sink treatments on grain Zn concentration and content of two wheat cultivars in the pot experiment.

Cultivars	Treatments		Zn concentration	Zn content
			mg kg^{-1}	µg pot^{-1}
Kenong 9204	T1	Deionized water	21.1c	345c
	T2	Sucrose	16.3c	255cd
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	49.6b	770b
	T4	Sucrose + Zn	65.7a	1092a
	T5	Partial spike removal	20.4c	373c
	T6	Spike shading	14.0c	193d
	T7	Defoliation	16.9c	147d
Liangxing 99	T1	Deionized water	14.2d	213c
	T2	Sucrose	24.2c	347c
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	38.5b	649b
	T4	Sucrose + Zn	59.0a	948a

Values are means of four and three replicates for 'Kenong 9204' and 'Liangxing 99', respectively.
Values in the same column per cultivar followed by different lower-case letters are significantly different among different treatments according to Fisher's Protected LSD test ($P \leq 0.05$).

Table 4. Effects of different source/sink manipulations on grain yield traits, grain Zn concentration and content of wheat 'Liangxing 99' in the field experiment at Quzhou.

Treatments	Yield	TKW	Zn concentration	Zn content
	t ha^{-1}	g	mg kg^{-1}	g ha^{-1}
T1 Deionized water	6.5a	37.6b	35.0bc	228.0bc
T2 Sucrose	6.2a	38.0b	30.8c	191.1c
T3 $ZnSO_4 \cdot 7H_2O$ (Zn)	6.4a	35.4bc	38.7ab	246.7ab
T4 Sucrose + Zn	6.4a	36.8bc	42.2a	271.6a
T5 Partial spike removal	–	45.5a	34.9bc	–
T6 Spike shading	–	29.5d	33.9bc	–
T7 Defoliation	–	30.6d	33.0bc	–

TKW: Thousand kernels weight.
Values in the same column followed by different lower-case letters are significantly different among various source/sink treatments according to Fisher's Protected LSD test ($P \leq 0.05$).

and T7) had nonsignificant effects on grain Zn concentrations. In addition, compared with T1, Zn-only treatment and sucrose + Zn increased the grain Zn content (Table 4).

Yield, grain Zn traits, and molar ratios of grain PA/Zn and PA × Ca/Zn of 'Jinan 17', 'Jimai 20', 'Jimai 22' and 'Luyuan 502' under field conditions at Yinmaquan

Wheat cultivars differed in TKW, grain Zn concentrations and contents, and molar ratios of PA/Zn and PA × Ca/Zn (Table 5). On average, the high-yielding 'Jimai 22' and 'Luyuan 502' had higher TKW, grain Zn contents, and molar ratios of PA/Zn and PA × Ca/Zn than 'Jimai 20' and 'Jinan 17'. 'Jinan 17', as a high-quality strong gluten wheat cultivar, had the lowest TKW and molar ratios of PA/Zn and PA × Ca/Zn. 'Jimai 20' had significantly lower grain Zn concentration and content than other cultivars. On average, the grain Zn concentration was significantly increased from the initial 40.9 to 51.6 mg kg^{-1} by Zn-only treatment and significantly increased further from 51.6 to 56.2 mg kg^{-1} by sucrose + Zn. Similar results were found in grain Zn contents. In contrast, T3 and T4 decreased molar ratios of PA/Zn by 20.1% and 29.6%, respectively, and decreased molar ratios of PA × Ca/Zn by 21.8% and 30.1%, respectively.

Pearson correlations among grain Zn concentration and other related parameters

Grain Zn concentration positively correlated with grain Zn content (Tables 6 and 7). There was significant and positive correlation between grain Zn concentration/content and TKW under field conditions (Table 7), however, this phenomenon was not observed in the pot experiment (Table 6). Grain Zn concentration negatively correlated with PA/Zn and PA × Ca/Zn and Zn content negatively correlated with PA/Zn, indicating the higher grain Zn concentration represented corresponding higher grain Zn bioavailability (Table 7). Positive correlations between grain yield and TKW or grain Zn content were found in both pot and field experiments (Tables 6 and 7). TKW positively correlated with PA/Zn and PA × Ca/Zn, respectively (Table 7).

Table 5. Effects of different foliar applications on grain yield, thousand kernel weight (TKW), grain Zn concentration and content, and molar ratios of grain phytic acid (PA)/Zn and PA × Ca/Zn of four different winter wheat cultivars in the field experiment at Yinmaquan.

			Winter wheat cultivars						
Parameters		Treatments	Jinan 17	Jimai 20	Jimai 22	Luyuan 502	Mean	*P*	
Yield, t ha^{-1}	T1	Deionized water	7.4a	7.4a	7.3a	7.5a	7.4A	C	0.6279
	T3	$ZnSO_4\cdot 7H_2O$ (Zn)	6.8a	7.0a	7.7a	6.9a	7.1A	T	0.5284
	T4	Sucrose + Zn	7.1a	7.0a	7.3a	7.3a	7.2A	C × T	0.7625
		Mean	**7.1A**	**7.1A**	**7.5A**	**7.2A**			
TKW, g	T1	Deionized water	40.9a	43.1ab	46.8a	49.5a	45.1A	C	< 0.0001
	T3	$ZnSO_4\cdot 7H_2O$ (Zn)	40.2a	44.2a	45.2a	48.5a	44.5A	T	0.6803
	T4	Sucrose + Zn	44.2a	42.6b	45.9a	48.4a	45.3A	C × T	0.3823
		Mean	**41.8C**	**43.3BC**	**46.0B**	**48.8A**			
Grain Zn concentrations, mg kg^{-1}	T1	Deionized water	40.0b	34.9b	42.9c	45.8c	40.9C	C	< 0.0001
	T3	$ZnSO_4\cdot 7H_2O$ (Zn)	51.8a	48.5a	51.7b	54.6b	51.6B	T	< 0.0001
	T4	Sucrose + Zn	59.7a	50.5a	56.3a	58.4a	56.2A	C × T	0.3904
		Mean	**50.5A**	**44.6B**	**50.3A**	**52.9A**			
Grain Zn contents, g ha^{-1}	T1	Deionized water	296b	257b	312b	342b	302C	C	0.0043
	T3	$ZnSO_4\cdot 7H_2O$ (Zn)	353ab	340a	401a	376ab	368B	T	< 0.0001
	T4	Sucrose + Zn	427a	351a	412a	424a	404A	C × T	0.6160
		Mean	**359A**	**316B**	**375A**	**381A**			
PA/Zn	T1	Deionized water	16.7a	18.1a	24.1a	20.8a	19.9A	C	0.0016
	T3	$ZnSO_4\cdot 7H_2O$ (Zn)	15.0a	15.2b	18.2b	15.3b	15.9B	T	< 0.0001
	T4	Sucrose + Zn	12.3a	13.3b	16.6b	13.6b	14.0C	C × T	0.5519
		Mean	**14.7B**	**15.5B**	**19.7A**	**16.5B**			
PA × Ca/Zn, mmol kg^{-1}	T1	Deionized water	157a	200a	310a	248a	229A	C	0.0002
	T3	$ZnSO_4\cdot 7H_2O$ (Zn)	146a	171b	236b	164b	179B	T	< 0.0001
	T4	Sucrose + Zn	124a	153b	205b	158b	160B	C × T	0.2980
		Mean	**142C**	**175B**	**250A**	**190B**			

Values in the same column per parameter followed by different lower-case letters are significantly different among various foliar treatments according to Fisher's Protected LSD test ($P \leq 0.05$).
Values of each parameter followed by different capital letters are significantly different among different wheat cultivars (horizontal comparison) or among various foliar treatments (vertical comparison) according to Fisher's Protected LSD test ($P \leq 0.05$).

Table 6. Pearson correlation coefficients among grain Zn concentration and other related parameters across all cultivars ('Kenong 9204' and 'Liangxing 99') in the pot experiment.

Parameters	Zn concentration	Grain yield	Biomass yield	TKW	Kernels per spike	HI	Zn content
Zn concentration	–	0.307	0.344	0.176	0.245	0.161	0.985***
Grain yield	–	–	0.987***	0.779***	0.499**	0.840***	0.453*
Biomass yield	–	–	–	0.750***	0.533**	0.747***	0.486*
TKW	–	–	–	–	-0.146	0.716***	0.287
Kernels per spike	–	–	–	–	–	0.295	0.320
Harvest index (HI)	–	–	–	–	–	–	0.287
Zncontent	–	–	–	–	–	–	–

TKW: Thousand kernels weight.
*, **, *** Significant correlations at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 7. Pearson correlation coefficients among grain Zn concentration and other related parameters across all cultivars under two field conditions.

Parameters	Zn concentration	Grain yield	TKW	Zn content	PA/Zn	PA × Ca/Zn
Zn concentration	–	0.205	0.558^{***}	0.898^{***}	-0.456^{***}	-0.306^{*}
Grain yield	–	–	0.427^{***}	0.609^{***}	0.122	0.102
TKW	–	–	–	0.590^{***}	0.308^{*}	0.377^{**}
Zn content	–	–	–	–	-0.338^{*}	-0.215
Phytic acid (PA)/Zn	–	–	–	–	–	0.946^{***}
PA × Ca/Zn	–	–	–	–	–	–

TKW: Thousand kernels weight.
*, **, *** Significant correlations at the 0.05, 0.01, and 0.001 probability levels, respectively.

DISCUSSION

This study showed that partial spike removal enlarged the grain sink, resulting in higher grain yield, biomass and TKW, without affecting the grain Zn concentration (Tables 2-4). TKW positively correlated with grain Zn concentration and content under field conditions but not in the pot experiment (Tables 6 and 7), which need to be further studied in the future. The reduction in photosynthetic source by shading spikes and especially by defoliation substantially decreased grain yield, biomass, grain size, and harvest index of wheat. Similar results were reported (Zhang et al., 2012a; Liu et al., 2014). Zhang et al. (2012a) showed that reducing source (through defoliation and spike shading) or sink (through removal of 50% spikelets) increased wheat grain Zn concentrations under field conditions. However, there was a trend that spike-shading and defoliation decreased wheat grain Zn concentrations, compared with control, under both controlled environment and field conditions (Tables 3 and 4). The grain Zn content of ‘Kenong 9204’ was reduced due to reduced grain weights in defoliation and spike shading. Similar results were found by Zhang et al. (2012a) that defoliation, spike shading and removal of 50% spikelets decreased grain Zn contents. The present study suggests that reducing photosynthetic source by defoliation and spike-shading changes source-sink relationships, decreases wheat grain weights, and further reduces grain Zn accumulation.

It has been reported that increased sucrose supply within the optimal level substantially enhanced grain yield and single grain weight of rice and wheat under detached-ear culture (Sasaki et al., 2005; Zhang et al., 2012a; Liu et al., 2014). Sasaki et al. (2005) speculated that the increase was due to the improved activities of enzymes involved in starch synthesis. However, in both pot and field experiments of our study, sucrose spraying with or without Zn had nonsignificant effects on grain yield components and harvest index (Tables 2, 4 and 5), indicating that DM accumulation in grains is less affected by external carbohydrate supply. It was shown that ^{65}Zn was not transported within the grain in the same way as ^{14}C-sucrose (Pearson et al., 1996a). Our study showed the foliar spray of only sucrose had nonsignificant effect on grain Zn contents in wheat (Tables 3 and 4). However, its effect on grain Zn concentrations varied for different cultivars (Table 3).

Foliar Zn spraying with or without sucrose did not affect yield traits of wheat (Tables 2, 4 and 5). Similar results were previously reported (Cakmak et al., 2010a; 2010b; Wang et al., 2012; Zhang et al., 2012b; Zhao et al., 2014). These results might be attributed to the relatively high Zn availability in the soils studied and thus the high plant Zn nutritional status. In agreement with others (Cakmak et al., 2010a; Wang et al., 2012; Zhang et al., 2012b), foliar Zn supply alone significantly increased grain Zn accumulation irrespective of wheat cultivars and environmental conditions in this study (Tables 3-5). Remarkably, a synergistic ‘sucrose + Zn’ spraying increased grain Zn more than Zn alone. Clearly, the foliar spray of ‘sucrose + Zn’ had achieved the grain Zn concentrations close to or more than the biofortification target value of 60 mg kg^{-1} according to the World Health Organization (WHO) report in 2006 (WHO, 2006). The relatively higher effectiveness of ‘sucrose + Zn’ can be attributed to a longer drying time of the Zn solution and improved leaf cuticle penetration and/or translocation rates of Zn from the site of absorption to grains, as suggested by Zhao et al. (2014). Further studies are needed to identify whether these processes are all manipulated by sucrose and ascertain their relative contributions to increased grain Zn accumulation.

In other studies, the effectiveness of foliar Zn application on the grain Zn concentration is related to the spray timing, location, rate and environmental conditions (Cakmak et al., 2010a; 2010b; Zhang et al., 2012b). Our study showed the grain Zn concentration of 'Liangxing 99' in 'sucrose + Zn' under field conditions was 28.5% lower than in the pot experiment. In order to further enhance Zn absorption and translocation to grains to achieve the target value of 60 mg kg^{-1} in field, appropriately increasing spraying times and the Zn/sucrose concentration and/or spraying with other fertilizers (e.g. N) might be beneficial (Zhao et al., 2014), which deserves further study.

In this current study, the remarkable decrease in molar ratios of PA/Zn and PA × Ca/Zn by foliar Zn supply with or without sucrose suggests the potentially increased grain Zn bioavailability (Table 5). The molar ratio of PA × Ca/Zn below the critical ratio of 200 indicated a good Zn bioavailability (Ellis et al., 1987). The sucrose + Zn treatment led to a relatively higher Zn bioavailability than the Zn-only treatment. Especially, molar ratios of PA/Zn of 'Jinan 17', 'Jimai 20' and 'Jimai 22' were reduced by sucrose + Zn treatment to less than 15, representing about 35% Zn availability according to the WHO report on trace elements in human nutrition and health (WHO, 1996). Cultivars obviously differed in their molar ratios of PA/Zn and PA × Ca/Zn. 'Jinan 17', as a high-quality strong gluten wheat cultivar, had a higher grain Zn bioavailability than other three wheat cultivars. Grain Zn bioavailability of the most commonly used high-yielding 'Jimai 22' in China was the lowest with molar ratios of PA/Zn and PA × Ca/Zn higher than the critical value of 15 and 200, respectively.

CONCLUSIONS

In this study, reducing plant inner carbohydrate sources by spike shading and defoliation changed the source-sink relationship and decreased grain Zn accumulation. Enlarging grain size or sink by removing stunting spikes had no obvious effects on grain Zn concentration. Foliar Zn spray is an effective way for biofortification of wheat grains with Zn. A synergistic external foliar supply of carbohydrate (sucrose) and Zn source is more effective than Zn-only spray in increasing grain Zn concentration and bioavailability. Mechanisms in relation to external sucrose supply (with or without Zn) influencing grain Zn accumulation need to be elucidated in the future.

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